SUPPLEMENTARY FIGURES

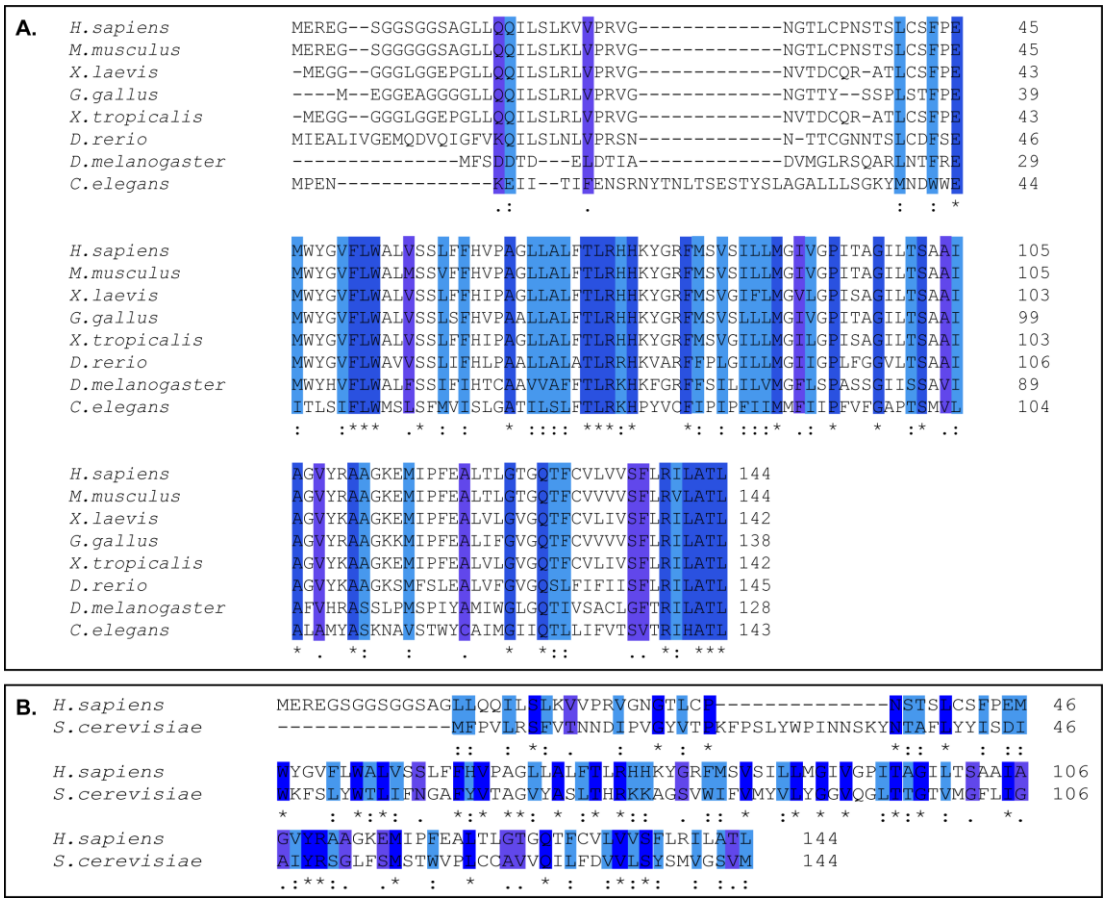


Fig. S1. TMEM170A is conserved in major eukaryotic phyla.

(A) Multiple sequence alignment of TMEM170A homologues from representative species.

(B) Sequence alignment of human TMEM170A and *S.cerevisiae* TMEM170A. The alignment was generated with Clustal Omega. Fully conserved amino acids were highlighted in dark blue and indicated by a star (∗) at the bottom, amino acids with strongly similar properties highlighted in light blue and indicated by a colon (:) and those with weakly similar properties with purple and indicated by a period (.). The following species and protein accession numbers from UniProtKB/Swiss-Prot database were used: Q8WVE7 (*Homo sapiens*); Q9D342 (*Mus musculus*); Q6DF87 (*Xenopus laevis*); F1N8V4 (*Gallus gallus*); A9ULP1 (*Xenopus tropicalis*); A3KPL7 **(***Danio rerio*); Q7JV61 **(***Drosophila melanogaster*); Q95QG3 **(***Caenorhabditis elegans***);** YPR153W **(***Saccharomyces cerevisiae***).**

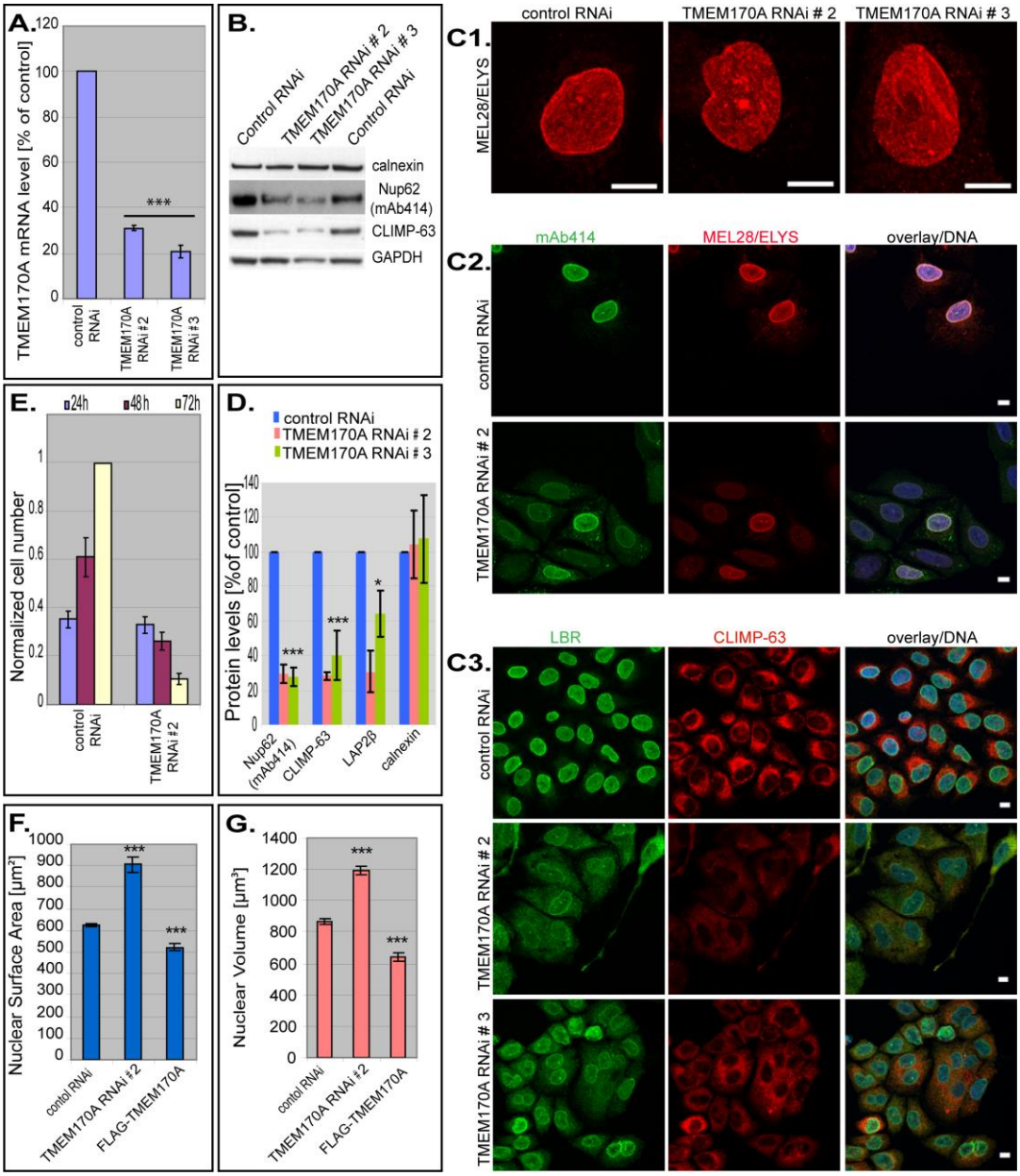


Fig. S2.

(A) Establishing the conditions for TMEM170A silencing.

Quantitative real-time PCR was used to measure mRNA levels of TMEM170A in TMEM170A silencing experiments using two different siRNA oligos (Table S3).

With siRNA oligo set #2, TMEM170A mRNA was reduced to 31.0 ± 1.3% (s.d.) of control-silenced levels where as with siRNA oligo set #3 TMEM170A mRNA was reduced to 20.6 ± 2.7% (s.d.) of control-silenced levels. mRNA levels of the *MGB2* housekeeping gene were used for sample normalization. n=3 independent experiments. Oligo set #2 was employed for further analysis. The *** indicate that the difference is extremely significant ($P < 0.001$).

(B) TMEM170A siRNA (oligo set #2 or oligo set #3) reduces protein levels of CLIMP-63 and nucleoporin Nup62 while levels of calnexin were unaffected in HeLa K total cell protein extracts from control and TMEM170A-silenced cells, as assayed by Western blot.

(C1-C2) siRNA knock-down of TMEM170A with siRNA oligo set #2 or oligo set #3 reduced nuclear rim signal in HeLa K cells. HeLa K cells were transfected with negative control or

TMEM170A siRNAs (oligo set #2 or oligo set #3) and 72 or 48 hr (respectively) post transfection and cells were fixed and stained with mAb414 (green), MEL-28/ELYS (red) antibodies. Nuclei are counterstained with Hoechst (blue). Scale bars: 10 µm.

(C3) TMEM170A-silenced cells with siRNA oligo set #2 or oligo set #3 were stained with CLIMP-63 antibody (red) and LBR (green). LBR (green) is strongly mislocalized to the ER in TMEM170A-silenced cells, compared to control cells, while silenced cells, stained with CLIMP-63 antibody (red), showed a reduced ER sheet signal, relative to controls. Nuclei were counterstained with Hoechst (blue). Scale bars: 10 µm.

(D) Quantitation by Western blot of Nup62, CLIMP-63, LAP2β and calnexin protein levels in control, TMEM170 RNAi #2-silenced and TMEM170 RNAi #3-silenced samples. GAPDH in the same sample was used as a loading control for sample normalization. Results are shown as average normalized value of each protein level as a percentage of the control value (set at 100%; n=3 independent Western blots). The *** and * indicate that the difference is extremely significant ($P < 0.001$) and significant ($P < 0.05$), respectively.

Results show a significant reduction of Nup62 (mAb414) protein levels in TMEM170 RNAi #2-silenced [to 29.49 ± 5.24 of control (P= 0.0009, Student's t-test)] and TMEM170 RNAi #3-silenced [to 27.62 ± 5.74 of control (P= 0.0005, Student's t-test)] samples. Results also show a significant reduction of CLIMP-63 protein levels in TMEM170 RNAi #2-silenced [to 28.47 ± 2.25 of control (P= $6.89*10^{-5}$, Student's t-test)] and TMEM170 RNAi #3-silenced [to 40.18 ± 13.91 of control (P= 0.0008, Student's t-test)] samples. Finally, LAP2β protein levels are also reduced in TMEM170 RNAi #2-silenced [to 30.73 ± 12.42 of control (P= 0.02, Student's t-test)] and TMEM170 RNAi #3-silenced to [62.42 ± 25.44 of control (P= 0.04, Student's t-test)] samples. Protein levels of calnexin were not reduced upon TMEM170A silencing.

(E) Down regulation of TMEM170A by siRNA causes cell death.

HeLa K cells were transfected with control or TMEM170A siRNA (oligo set #2) and cells were counted after 24 hr, 48 hr and 72 hr. Cell numbers were normalized to 72 hr control siRNA cell numbers. Average of three independent experiments, for each time-point. Error bars show S.D.

(F) Quantitation of nuclear surface area of control-silenced cells, TMEM170A-silenced cells or transiently transfected with FLAG-TMEM170A HeLa K cells, n=52 cells per condition from three independent experiments. Error bars show S.D. The *** indicate that the difference is extremely significant ($P < 0.001$).

TMEM170A-silenced cells showed an increase of their nuclear surface area to 145.68 ± 4.82% compared to control cells [622.21 ± 6.87 μm^2 in controls *vs.* 906.58 ± 36.52 μm^2 in silenced cells (*P*= 0.00019, Student's t-test)] whereas FLAG-TMEM170A transfected cells showed a reduction of nuclear surface area to 83.9 ± 1.9% of control cells to control cells [622.21 ± 6.87 μm^2 in controls *vs.* 522.14 ± 15.88 μm^2 in silenced cells (*P*= 0.0005, Student's t-test)].

(G) Quantitation of nuclear volume of control-silenced cells, TMEM170A-silenced cells or transiently transfected with FLAG-TMEM170A HeLa K cells, n=52 cells per condition from three independent experiments. Error bars show S.D. The *** indicate that the difference is extremely significant ($P < 0.001$).

TMEM170A-silenced cells showed an increase of their nuclear volume to 137.38 ± 1.13% of control cells [867.91 ± 16.51 μm^3 in controls *vs.* 1192.4 ± 28.39 μm^3 in silenced cells (*P*= $6.84*10^{-5}$, Student's t-test)] whereas FLAG-TMEM170A transfected cells showed a reduction of nuclear volume to 73.97 ± 19.82% control cells [867.91 ± 16.51 μm^3 in controls *vs.* 643.31 ± 25.47 μm^3 in FLAG-TMEM170A overexpressing cells (*P*= 0.0002, Student's t-test)].

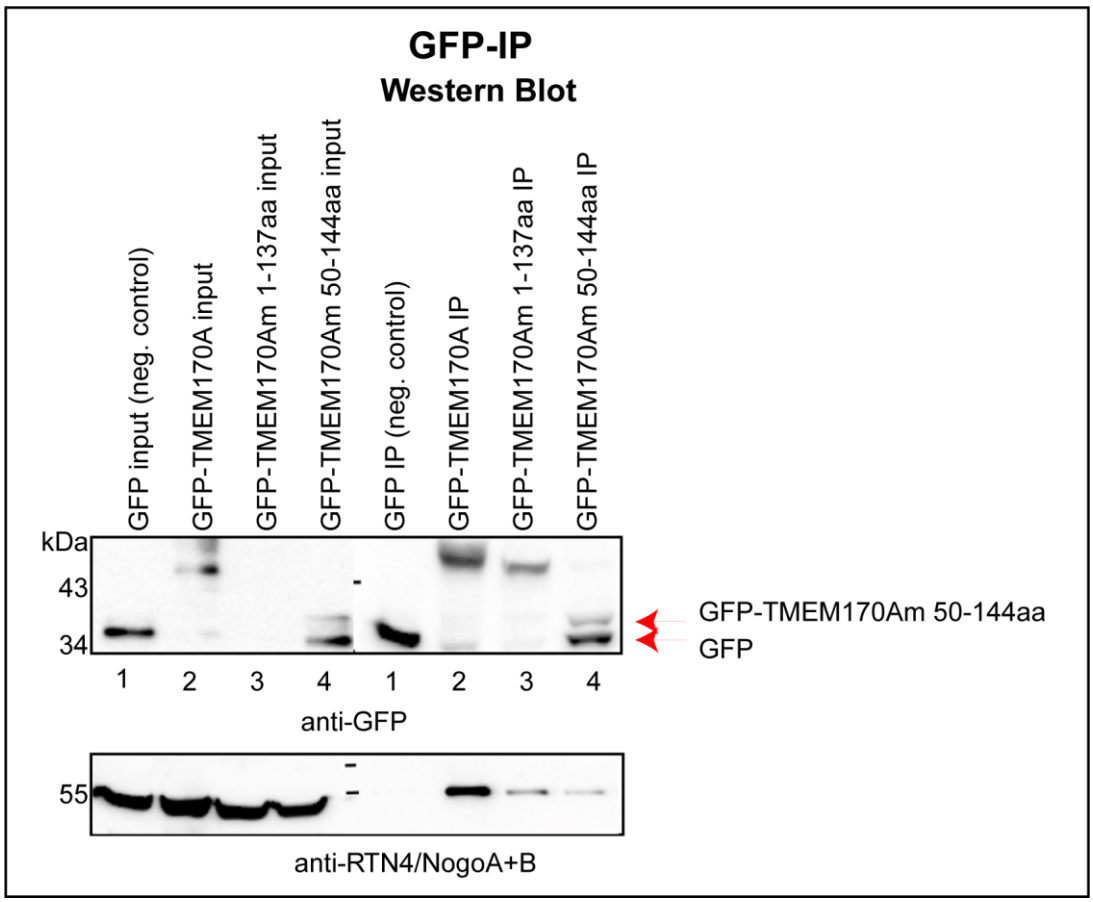


Fig. S3. Western blot analysis indicating that the N- and C-termini of TMEM170A are not required for the interaction with RTN4.

Full-length TMEM170A (lane 2) and two TMEM170A mutants were fused with a GFP tag at their C-terminus. One of the mutants was missing the first 49 N-terminal amino acids (TMEM170Am_50-144) (lane 4) and the second was missing the last C-terminal seven amino acids (TMEM170Am_1-137) (lane 3). “Input” corresponds to 1/40 volume of the lysate used for the reaction and “IP” is 1/2 of the bound fraction (i.e. protein complexes captured on the beads).

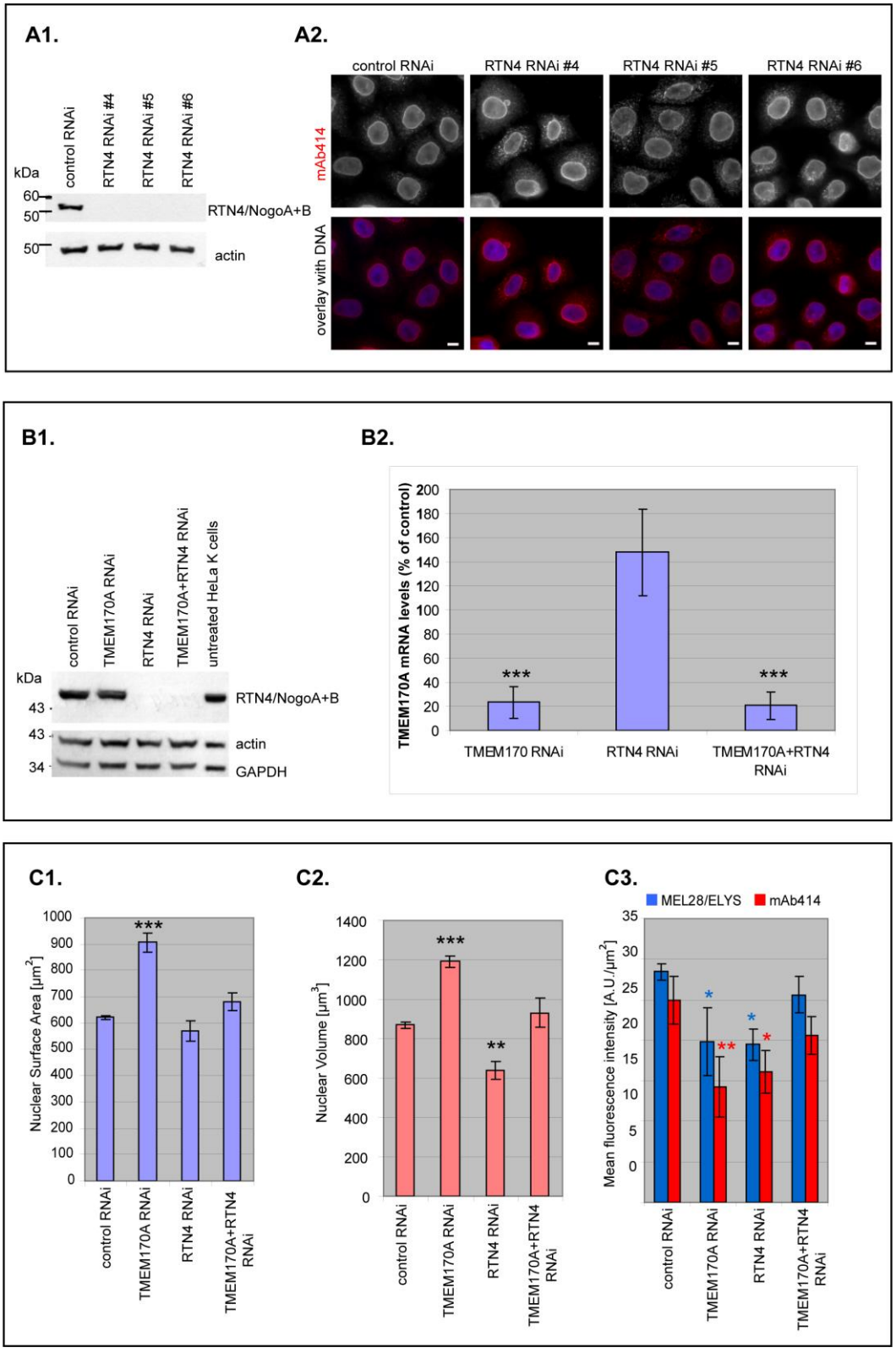


Fig. S4. Establishing conditions for RTN4 and double TMEM170A+RTN4 silencing.

(A1) Efficiency of RTN4 silencing by Western blot. Three siRNA oligos for RTN4 were tested (Table S3).

(A2) RTN4-silenced cells, stained with mAb414 (red), showed reduced nuclear rim signal and mis-localizaton to the cytoplasm, relative to control cells. Oligo set #5 gave the strongest phenotype and was employed for further analysis. Nuclei are counterstained with Hoechst (blue). Scale bars: 10 µm.

(B1) Efficiency of RTN4 silencing by Western blot in single TMEM170A or RTN4 and TMEM170A plus RTN4 co-silencing.

(B2) Quantitative real-time RT-PCR was used to measure mRNA levels of TMEM170A in single TMEM170A or RTN4 or double TMEM170A plus RTN4 silencing experiments. In single TMEM170A silencing, TMEM170A mRNA was reduced to 23.45 ± 13% (S.D.) of control-silenced levels. In single RTN4 silencing, TMEM170A mRNA is not affected [(147.82 ± 36)% (S.D.) of control-silenced levels]. Finally, in double TMEM170A plus RTN4 silencing, TMEM170A mRNA was reduced to 20.46 ± 11% (S.D.) of control-silenced levels. mRNA levels of the *MGB2* housekeeping gene were used for sample normalization. N=3 independent experiments. The *** indicate that the difference is extremely significant ($P < 0.001$).

Double TMEM170A plus RTN4 silencing restores nuclear size and NPC density, affected either by single TMEM170A or RTN4 silencing in HeLa K cells

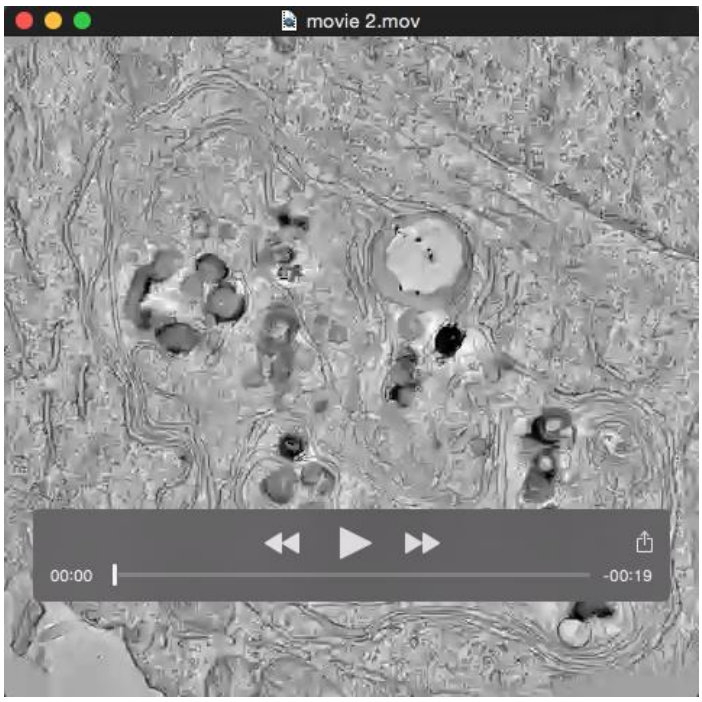
(C1) Quantitation of nuclear surface area of HeLa K transfected with control or TMEM170A or RTN4 and TMEM170A + RTN4 co-silencing. Shown is the average of three independent experiments, $n > 52$ cells (for each condition per experiment). Error bars show S.D. The *** indicate that the difference is extremely significant ($P < 0.001$).

(C2) Quantitation of nuclear volume of HeLa K transfected with control or TMEM170A or RTN4 and TMEM170A + RTN4 co-silencing. Shown is the average of three independent experiments, $n > 52$ cells (for each condition per experiment). Error bars show S.D. *** and ** indicate that the difference is extremely significant ($P < 0.001$) and very significant ($P < 0.01$), respectively.

(C3) Quantitation of mean immunofluorescence MEL28/ELYS and mAb414 intensity to estimate NPC density in HeLa K cells transfected with control or TMEM170A or RTN4 and TMEM170A + RTN4 co-silencing. $n > 52$ nuclei per condition from three independent experiments. Error bars show S.D. ** and ** indicate that the difference is very significant ($P < 0.01$) and significant ($P < 0.05$), respectively.

SUPPLEMENTARY VIDEOS

Movie 1: 3D electron tomography in TMEM170A-silenced HeLa K cells.
TMEM170A silencing-induced structures that appear to be, at first glance, well-organized structure of smooth ER; however, 3D scanning through the movie demonstrates that the tubules are mostly still separated into small sections rather than joined up into cisternal stacks.



Movie 2: 3D electron tomography in FLAG-TMEM170A overexpressing cells HeLa K cells.

Overexpression of FLAG-TMEM170A induced well-organized and extensive ER sheet stacks.

Table S1. List of proteins that co-immunoprecipitated with TMEM170A (Fig. 7; bands 1-3) as identified by mass spectrometric analysis.

Band	Accession No.	Protein Name
1	FAS_HUMAN GCN1L_HUMAN	Fatty acid synthase Translational activator GCN1
2	RTN4_HUMAN	Reticulon-4
3	RAN_HUMAN	GTP-binding nuclear protein Ran

Table S2. List of oligonucleotides used for cloning and quantitative RT-PCR

Set	Forward primer 5' → 3'	Reverse primer 5' → 3'
1	TGTGCCCCAACTCTACTTCC	AGTGCCCACAGGAATACACC
2	AGC GTA CTC CAA AGA TTC AGG TT	TAC ATG TCT CGA TCC CAC TTA ACT AT
3	CGCTCGAGATGGAGCGCGAGGGGAGCGGCGGCA	CGGAATTCGTAGAGTAGCTAAAATCCGTAAAAAGG
4	CGGAATTCATGGAGCGCGAGGGGAGCGGCGGCA	GCGCTCGAGGTAGAGTAGCTAAAATCCGTAAAAAGG
5	CGCGCTCGAGTTCCTGTGGGCACTGGTGTCTTCTCTC	CGGAATTCGTAGAGTAGCTAAAATCCGTAAAAAGG
6	CGCGCTCGAGATGGAGCGCGAGGGGAGCGGCGGCA	CGGAATTCGAAAGGAGACCACCAAGACGCA

Table S3. A list of siRNA oligonucleotides used.

Set	Company	Name	Sequence (5' → 3')
1	MWG	Luciferase siRNAs –Negative control oligo	UCGAAGUAUUCCGCGUACG
2	Ambion	TMEM170A (141393)	GGACAACACAUCUCAGCCU
3	Ambion	TMEM170A (38236)	GGAACAUGAAGGACAACAC
4	Ambion	RTN4 (6828)	GGGCAUAUCUGGAAUCUGA
5	Ambion	RTN4 (289847)	CCAUCAGCUUUAGGAUAUA
6	Ambion	RTN4 (6738)	GGGUGUGAUCCAAGCUAUC

Highlighted in blue are the ones mainly used for this work.

TABLE S4. Primary and secondary antibodies used in this work

PRIMARY ANTIBODIES (TO)	SOURCE/REFERENCE	WORKING DILUTION
mAb414 mouse mab	Covance, MMS-120P	1:1000 (IF&WB)
Pom121 rabbit ab	Yavuz et al., 2010	1:20 (IF)
MEL28/ELYS rabbit ab	Franz et al., 2007	1:2000 (IF&WB)
RTN4/NogoA+B rabbit ab	Abcam, ab47085	1:300 (IF&WB)
LBR (E398L) rabbit mab	Abcam, ab32535	1:300 (IF&WB)
LAP2β mouse ab	BD Transduction Laboratories, 611000	1:500 (IF&WB)
emerin rabbit ab	Abcam ab14208	1:300 (IF&WB)
calnexin (H-70) rabbit ab	Santa Cruz, sc-11397	1:100 (IF&WB)
calreticulin (H-170) rabbit ab	Santa Cruz, sc-11398	1:50 (IF)
FLAG mouse mab	Sigma-Aldrich, F4042	1:500 (IF)
lamin A (133A2) mouse mab	Abcam ab8980	1:500 (IF)
LEM4 rabbit ab	Asencio et al., 2012	1:100 (IF)
CLIMP-63 (G1/296) mouse mab	Enzo, ALX-804-604-C100	1:1000 (IF&WB)
Nup160 rabbit ab	Abcam, ab74147	1:500 (WB)
GFP rabbit ab	Yavuz et al., 2010	1:1000 (IF)
GFP mouse ab	Roche, 11814460001	1:1000 (WB)
Actin (AC-74) mouse mab	Sigma-Aldrich, A2228	1:10000 (WB)
GAPDH (6C5) mouse mab	Santa Cruz sc-32233	1:500 (WB)
SECONDARY ANTIBODIES	**SOURCE**	**WORKING DILUTION**
AF350 goat anti-mouse IgG	Molecular Probes A11045	1:100 (IF)
AF488 goat anti-mouse IgG	Molecular Probes A11017	1:1500 (IF)
AF488 goat anti-rabbit IgG	Molecular Probes A11034	1:1500 (IF)
AF488 donkey anti-mouse IgG	Molecular Probes A21202	1:1000 (IF)
AF488 donkey anti-rabbit IgG	Molecular Probes A21206	1:1000 (IF)
AF568 goat anti-mouse IgG1	Molecular Probes A21124	1:1500 (IF)
AF568 goat anti-rabbit IgG	Molecular Probes A11036	1:1500 (IF)
AF555 donkey anti-rabbit IgG	Molecular Probes A31572	1:1000 (IF)
AF555 donkey anti-mouse IgG	Molecular Probes A31570	1:1000 (IF)
HRP donkey anti-rabbit IgG	Santa Cruz sc-2077	1:20,000 (WB)
HRP sheep anti-mouse IgG	GE Healthcare NA931	1:8000 (WB)

DNA was counter stained with 0.5 µg/ml Hoechst 33342.

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